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=> file medline hcaplus biosis biotechds scisearch embase
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FULL ESTIMATED COST                               0.21       0.21

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=> s modulating I-kappa kinase
L1             0 MODULATING I-KAPPA KINASE

=> s  I-kappa kinase
L2            39 I-KAPPA KINASE

=> dup rem l2
PROCESSING COMPLETED FOR L2
L3            26 DUP REM L2 (13 DUPLICATES REMOVED)

=> s l3 and cellular localization
L4             0 L3 AND CELLULAR LOCALIZATION

=> s l3 and cellular
L5             2 L3 AND CELLULAR

=> d l5 1-2 ibib ab

L5   ANSWER 1 OF 2  BIOSIS  COPYRIGHT (c) 2004 The Thomson Corporation.  on STN
ACCESSION NUMBER:   2002:219617  BIOSIS
DOCUMENT NUMBER:    PREV200200219617
TITLE:              Heat shock inhibits activation of NF-kappaB in the absence
                    of heat shock factor-1.
AUTHOR(S):          Malhotra, Vivek; Eaves-Pyles, Tonyia; Odoms, Kelli; Quaid,
                    Gina; Shanley, Thomas P.; Wong, Hector R. [Reprint author]
CORPORATE SOURCE:   Division of Critical Care Medicine, Children's Hospital
                    Medical Center, 3333 Burnet Avenue, OSB5, Cincinnati, OH,
                    45229-3039, USA
                    wonghr@chmcc.org
SOURCE:             Biochemical and Biophysical Research Communications, (March
                    1, 2002) Vol. 291, No. 3, pp. 453-457. print.
                    CODEN: BBRCA9. ISSN: 0006-291X.
DOCUMENT TYPE:      Article
LANGUAGE:           English
ENTRY DATE:         Entered STN: 27 Mar 2002
                    Last Updated on STN: 27 Mar 2002
AB   The heat shock response is known to inhibit NF-kappaB activation and
     NF-kappaB-dependent gene expression.  Herein we determined if cells
     lacking heat shock factor-1 (HSF-1), the major transcription factor
     regulating heat shock protein gene expression, have an altered ability to
     modulate NF-kappaB activation.  Embryonic fibroblasts from HSF-1-null
```

mutant mice (HSF-1-/- cells) had a drastically reduced ability to express heat shock protein-70 in response to heat shock, compared to embryonic fibroblasts from wild-type mice (HSF+/+ cells). There was no difference, however, in the ability of heat shock to inhibit TNFalpha-mediated NF-kappaB activation, IkappaBalpha degradation, IkappaB kinase activation, and macrophage chemotactic protein-1 expression in the HSF-1-/- cells compared to the HSF-1+/+ cells. These data demonstrate that heat shock inhibits activation of the NF-kappaB/IkappaBalpha pathway and NF-kappaB-dependent gene expression in the absence of an intact heat shock response.

L5 ANSWER 2 OF 2 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 2001:92225 BIOSIS
DOCUMENT NUMBER: PREV200100092225
TITLE: Intracellular delivery of proteins into endothelial cells by membrane permeable peptide vectors.
AUTHOR(S): Baranyi, U. [Reprint author]; Schwartz, C. [Reprint author]; Kroismayr, R. [Reprint author]; Binder, B. R. [Reprint author]; Lipp, J. [Reprint author]
CORPORATE SOURCE: Vascular Biology and Thrombosis Research, University of Vienna-VIRCC, Vienna, Austria
SOURCE: Journal of Submicroscopic Cytology and Pathology, (July, 2000) Vol. 32, No. 3, pp. 473. print.
Meeting Info.: XIth International Vascular Biology Meeting. Geneva, Switzerland. September 05-09, 2000.
ISSN: 1122-9497.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 21 Feb 2001
Last Updated on STN: 12 Feb 2002

=> s l3 and inhibitor
L6 7 L3 AND INHIBITOR

=> d l6 1-7 ibib ab

L6 ANSWER 1 OF 7 MEDLINE on STN
ACCESSION NUMBER: 2003137453 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12651903
TITLE: The effects of labour and of interleukin 1 beta upon the expression of nuclear factor kappa B related proteins in human amnion.
AUTHOR: Lee Yooni; Allport Victoria; Sykes Anna; Lindstrom Tamsin; Slater Donna; Bennett Phillip
CORPORATE SOURCE: Imperial College Parturition Research Group, Institute of Reproductive and Developmental Biology, Hammersmith Hospital Campus, DuCane Road, London W12 0NN.
SOURCE: Molecular human reproduction, (2003 Apr) 9 (4) 213-8.
Journal code: 9513710. ISSN: 1360-9947.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200309
ENTRY DATE: Entered STN: 20030325
Last Updated on STN: 20030926
Entered Medline: 20030925
AB Human labour is associated with persistently increased nuclear factor kappa B (NF-kB) activity in amnion. In this study we have shown that this involves only the p65 and p50 NF-kB subunits and is associated with an increase in the expression of p65 ($P < 0.05$), and of the NF-kB binding proteins IkBa, IkBb-1 and IkBb-2 ($P < 0.05$). Interleukin-1b stimulation leads to rapid degradation and resynthesis of IkBa within 2 h, and a

decrease in IkBb-1 without a return to full expression by 2 h, but has little effect upon IkBb-2. IkBb-2 was found in both the cytosolic and nuclear protein fractions. These findings demonstrate that persistently increased NF-kB activity in amnion occurs despite increased expression of the inhibitory IkBa protein and is not mediated by persistant **I-kappa kinase** activity or inhibition of IkBa synthesis.
The increased expression and nuclear localization of IkBb-2 suggests that its function may be to protect NF-kB from inactivation by IkBa and to maintain NF-kB-mediated gene transcription.

L6 ANSWER 2 OF 7 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2004:58767 HCAPLUS
DOCUMENT NUMBER: 140:247332
TITLE: The molecular mechanism of sensitization to Fas-mediated apoptosis by 2-methoxyestradiol in PC3 prostate cancer cells
AUTHOR(S): Shimada, Keiji; Nakamura, Mitsutoshi; Ishida, Eiwa; Kishi, Munehiro; Matsuyoshi, Syuchi; Konishi, Noboru
CORPORATE SOURCE: Department of Pathology, Nara Medical University, Nara, Japan
SOURCE: Molecular Carcinogenesis (2003), Volume Date 2004, 39(1), 1-9
CODEN: MOCAE8; ISSN: 0899-1987
PUBLISHER: Wiley-Liss, Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB It is widely known that death receptor Fas-dependent apoptotic signals are assocd. with development of prostate cancer, but the key pathways involved in sensitivity to the apoptosis remain unclear. Here the authors investigated the mol. mechanism by which 2-methoxyestradiol (2-ME) effectively sensitizes a human prostate cancer cell line, PC3, to Fas-mediated apoptosis. The 2-ME significantly inhibited nuclear factor-.kappa.B (NF-.kappa.B) activation and downregulated Fas-assocd. death domain (FADD) protein interleukin-1beta-converting enzyme inhibitory protein (FLIP). Overexpression of the dominant neg. mutant form of I.kappa.B.alpha. (d/n I.kappa.B.alpha.) or treatment with **I. kappa. kinase**-specific **inhibitor** Bay117082 gave the same results, although the sensitizing effect was not as pronounced. A selective **inhibitor** of Akt phosphorylation, LY294002, accelerated formation of the death-inducing signaling complex (DISC) not only by FLIP redn. but also by enhancement of recruitment of the FADD to Fas, thereby sensitizing PC3 cells to apoptosis similar to the case with 2-ME stimulation. Moreover, the authors found that inhibition of 2-ME-induced extracellular signalregulated kinase (ERK) activation by the upstream kinase **inhibitor** PD98059 significantly enhanced 2-ME-mediated suppression of Akt activation, resulting in much greater sensitization to apoptosis. Taken together, the present findings indicate that 2-ME suppresses NF-.kappa.B/FLIP signaling and enhances DISC formation through inhibition of Akt, and that PC3 cells thereby are being sensitized to Fas-mediated apoptosis and by a process closely assocd. with ERK.
REFERENCE COUNT: 46 THERE ARE 46 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 3 OF 7 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2003:1009436 HCAPLUS
DOCUMENT NUMBER: 140:91412
TITLE: Shear Stress Regulates Endothelial Nitric-oxide Synthase Promoter Activity through Nuclear Factor .kappa.B Binding
AUTHOR(S): Davis, Michael E.; Grumbach, Isabella M.; Fukai, Tohru; Cutchins, Alexis; Harrison, David G.
CORPORATE SOURCE: Division of Cardiology, Emory University, Atlanta, GA, 30322, USA
SOURCE: Journal of Biological Chemistry (2004), 279(1),

163-168
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB We have previously demonstrated that shear stress increases transcription of the endothelial nitric-oxide synthase (eNOS) by a pathway involving activation of the tyrosine kinase c-Src and extracellular signal-related kinase 1/2 (ERK1/2). In the present study sought to det. the events downstream of this pathway. Shear stress activated a human eNOS promoter chloramphenicol acetyl-CoA transferase chimeric construct in a time-dependent fashion, and this could be prevented by inhibition of the c-Src and MEK1/2. Studies using electromobility shift assays, promoter deletions, and promoter mutations revealed that shear activation of the eNOS promoter was due to binding of nuclear factor .kappa.B subunits p50 and p65 to a GAGACC sequence -990 to -984 base pairs upstream of the eNOS transcription start site. Shear induced nuclear translocation of p50 and p65, and activation of the eNOS promoter by shear could be prevented by co-transfection with a dominant neg. I kappa B.alpha.. Exposure of endothelial cells to shear resulted in **I.kappa.** **kinase** phosphorylation, and this was blocked by the MEK1/2 **inhibitor** PD98059 and the cSrc **inhibitor** PP1, suggesting these signaling mols. are upstream of NF.kappa.B activation. These expts. indicate that shear stress increases eNOS transcription by NF.kappa.B activation and p50/p65 binding to a GAGACC sequence present of the human eNOS promoter. While NF.kappa.B activation is generally viewed as a proinflammatory stimulus, the current data indicate that its transient activation by shear may increase expression of eNOS, which via prodn. of nitric oxide could convey anti-inflammatory and anti-atherosclerotic properties.

REFERENCE COUNT: 32 THERE ARE 32 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 4 OF 7 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2003:1003928 HCAPLUS
DOCUMENT NUMBER: 140:3799
TITLE: Mutations in murine transcription factor I.kappa.K.gamma. provide a model for incontinentia pigmenti and drug screening
INVENTOR(S): Makris, Konstantinos; Karin, Michael
PATENT ASSIGNEE(S): Regents of the University of California, USA
SOURCE: U.S. Pat. Appl. Publ., 28 pp.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2002056150	A1	20020509	US 2001-882507	20010615
US 6689564	B2	20040210		

PRIORITY APPLN. INFO.: US 2000-212438P P 20000616

AB The present invention relates to compns. and methods involving I.kappa.K.gamma. mutants. I.kappa.K.gamma. (also known as NF-.kappa.B essential modulator or NEMO) is required for NF-.kappa.B activation and resistance to tumor necrosis factor-induced apoptosis. Female mice heterozygous for I.kappa.K.gamma. deficiency develop unique dermatopathy, characterized by keratinocyte hyperproliferation, skin inflammation, hyperkeratosis, and increased apoptosis. The symptoms and inheritance pattern are very similar to those in incontinentia pigmenti (IP), a human genodermatosis that is syntenic with the I.kappa.K.gamma. locus. Biopsies and cells from IP patients exhibit defective expression but normal **I.kappa.** **kinase** catalytic subunits. Thus, the

present invention provides methods and compns., including transgenic animals, suitable for use in detg. means to treat, control, and/or prevent incontinentia pigmenti (IP), as well as a disease model for screening biol. active agents to treat IP. Methods to detect the presence of mutations in the I.kappa.K.gamma. gene and protein include immunoblot, Northern blot, Southern blot, RT-PCR, SSCP anal., and/or conformation-sensitive gel electrophoresis.

L6 ANSWER 5 OF 7 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 2003:459308 BIOSIS
DOCUMENT NUMBER: PREV200300459308
TITLE: The novel **inhibitor** of IKKbeta ameliorates insulin resistance and hyperglycemia.
AUTHOR(S): Kamon, Junji [Reprint Author]; Yamauchi, Toshimasa [Reprint Author]; Muto, Susumu [Reprint Author]; Waki, Hironori [Reprint Author]; Takekawa, Sato [Reprint Author]; Ito, Yusuke [Reprint Author]; Itai, Akiko [Reprint Author]; Kadowaki, Takashi [Reprint Author]
CORPORATE SOURCE: Tokyo, Japan
SOURCE: Diabetes, (2003) Vol. 52, No. Supplement 1, pp. A123. print.
Meeting Info.: 63rd Scientific Sessions of the American Diabetes Association. New Orleans, LA, USA. June 13-17, 2003. American Diabetes Association.
ISSN: 0012-1797 (ISSN print).
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 8 Oct 2003
Last Updated on STN: 8 Oct 2003

L6 ANSWER 6 OF 7 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 2002:386446 BIOSIS
DOCUMENT NUMBER: PREV200200386446
TITLE: Novel inhibitors of **I kappa kinase** and their functional significance in the treatment of breast cancer.
AUTHOR(S): Muthalif, Mubarack [Reprint author]; Kaspar, Allan [Reprint author]; Zapf, Jim [Reprint author]; Pham, Youm [Reprint author]; Vezza, Roberta [Reprint author]; Fanjul, Andrea [Reprint author]; Al-Shamma, Hussein [Reprint author]; Spruce, Lyle [Reprint author]; Zegelman, Lev [Reprint author]; Wiemann, Torsten [Reprint author]; Pfahl, Magnus [Reprint author]
CORPORATE SOURCE: Maxia Pharmaceuticals, Inc., San Diego, CA, USA
SOURCE: Proceedings of the American Association for Cancer Research Annual Meeting, (March, 2002) Vol. 43, pp. 200. print.
Meeting Info.: 93rd Annual Meeting of the American Association for Cancer Research. San Francisco, California, USA. April 06-10, 2002.
ISSN: 0197-016X.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 17 Jul 2002
Last Updated on STN: 17 Jul 2002

L6 ANSWER 7 OF 7 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-00172 BIOTECHDS
TITLE: Rapid high-throughput ion exchange resin assay for determining enzyme activity, comprises contacting enzymes with labeled substrate and coupling substrate or charged product to ion-exchange resin to separate substrate from charged product;
glutamine-fructose-6-phosphate-transaminase activity

determination using ionexchanger and modulator drug screening

AUTHOR: KARSTEN T P; CURRIE M G; MOORE W M
PATENT ASSIGNEE: KARSTEN T P; CURRIE M G; MOORE W M
PATENT INFO: US 2002072082 13 Jun 2002
APPLICATION INFO: US 2001-888008 22 Jun 2001
PRIORITY INFO: US 2001-888008 22 Jun 2001; US 2000-213354 22 Jun 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-589473 [63]

AB DERWENT ABSTRACT:

NOVELTY - Determining (M1) enzyme activity comprising contacting a compound such as enzymes, enzyme fragments or abzymes with a labeled substrate to form a differentially-charged product (DCP), selectively coupling either the substrate or DCP to an ion-exchange resin thus substantially separating the substrate from DCP, and determining the amount of substrate remaining or DCP formed using a measuring device, is new.

DETAILED DESCRIPTION - Determining (M1) enzyme activity comprising contacting a compound such as enzymes, enzyme fragments or abzymes with a labeled substrate to form DCP, selectively coupling either the substrate or DCP to an ion-exchange resin thus substantially separating the substrate from DCP, and determining the amount of substrate remaining or DCP formed using a measuring device. Optionally, M1 involves stopping the conversion before all of the substrate present is converted to DCP before the step of selectively coupling either the substrate or DCP to an ion-exchange resin. The stopping step and coupling step are carried out concurrently or sequentially. INDEPENDENT CLAIMS are included for the following: (1) determining (M2) bi-functional enzyme activity involves contacting an enzyme with a first labeled substrate to form a first DCP, contacting the enzyme with a second labeled substrate to form a second DCP, selectively coupling to an ion exchange resin, one or two members selected from the first substrate, the second substrate, the first product and the second product, thus substantially separating the member from the remaining member of the groups and determining the amount of the member using a measuring device; (2) a kit for determining enzyme activity, comprises an enzyme, a labeled ligand, a buffer solution, an ion-exchange resin or a stop-buffer solution; and (3) a compound discovered using M1.

WIDER DISCLOSURE - An integrated system for high throughput screening of potential modulators of enzyme activity is also disclosed.

BIOTECHNOLOGY - Preferred Method: In M1, the product or the substrate is bound to the resin. The product or substrate measured is in solution or is coupled to the resin. The enzyme is preferably a kinase. The method is conducted in a multiple-well format which comprises at least 96 wells. The format is automated. The high-throughput format is conducted on a microchip. In M2, the determination of bi-functional enzyme activity is conducted separately.

USE - M1 is useful for determining activity of enzymes such as glutamine:fructose-6-phosphate amidotransferase (GFAT), nitric oxide synthase, methionine aminopeptidase, Asn Syn, PFK, p38, **I-kappa kinase** 1, **I-kappa kinase** 2, TBK1, MAPKAP2, GTase, OGTase (all undefined) or cyclooxygenase. M1 is useful for identifying a molecule, compound or composition that affects the activity of an enzyme (in particular isozyme), for determining the kinetics of an enzyme reaction, for determining the functional sites on an enzyme and for evaluating the selective coupling of an enzyme and a reactant (claimed). M1 is useful for screening compounds or composition that selectively affect enzyme activity (e.g. specific **inhibitor** of an enzyme), for screening compounds (agonist, antagonist, mimetic or small molecule **inhibitor** of the isozyme) that promotes, reduces, irreversibly inhibits, or reversibly inhibits the isozyme's activity in a protein, fragment or fusion protein. M1 is also useful in bench top assays, in automated high-throughput screens, in automated system such as CRS A251

robotic system and ORCA integrated robotic system, in microchip system such as Caliper's LabChip high-throughput system platform or other combinations of matrix materials (e.g. silicates, cellulases) with programmable data storage or recording devices or other memory unit. M1 is also useful for rational drug design to produce structural analogs of biologically active polypeptides of interest or of small molecules with which they interact e.g. agonists, antagonists or inhibitors. M1 when combined with mutagenesis method is useful for detecting activity of cloned, mutagenized polypeptides in host cells, for determining the importance of individual amino acid residues in a polypeptide of interest and is applied to polypeptides of unknown structure, for predicting three-dimensional structure of a protein of interest or a protein **inhibitor** complex, for screening mutants homologs for functional activity and for identifying and/or for preparing a variety of polypeptides that are substantially homologous to the active site of an enzyme or their allelic variants.

ADVANTAGE - M1 has greater sensitivity. M1 is utilized in a variety of enzymatic reactions and the reaction is not hindered by the resin. M1 is easily automated because it is performed in a single step and is utilized to simultaneously assay the binding of more than one compound or ligand to an enzyme.

EXAMPLE - Activity of glutamine:fructose-6-phosphate amidotransferase (rhGFATI) was measured by separating the substrate, 14C-fructose-6-phosphate, from the product, 14C-glucosamine-6-phosphate using an anion exchange resin method. RhGFAT was over-expressed in insect cells using a baculovirus infection vector. Enzyme activity was identified in the cytosolic fraction and was purified partially by chromatography on DEAE-Sepharose. Identification of test substances was performed in an assay volume of 50 ml in a 96 well format. Enzyme (rhGFAT I) was added to initiate the assay containing 20 mM Imidazole pH 6.8, 1 mg/ml bovine serum albumin, 0.4 mM dithiotheritol (DTT), 10% glycerol, 10 mM KCl, 20 mM 14C-fructose-6-phosphate and 400 mM L-Glutamine. After 60 minutes of incubation, the assay was stopped by adding 150 ml of a suspension of Dowex AG1x8 anion exchange resin equilibrated in 10 mM sodium formate buffer pH 3.0. Unreacted 14C-fructose-6-phosphate was captured by the resin, whereas 14C-glucosamine-6-phosphate was unbound and remained in the buffer. The product was quantified by removing a 50 ml aliquot, scintillation cocktail of 200 ml was added and then counted in a Packard Topcount. (7 pages)

```
=> s IKK beta and GFP fusion
L7            0 IKK BETA AND GFP FUSION

=> s GFP fusion
L8         4799 GFP FUSION

=> dup rem l8
PROCESSING IS APPROXIMATELY  39% COMPLETE FOR L8
PROCESSING IS APPROXIMATELY  65% COMPLETE FOR L8
PROCESSING COMPLETED FOR L8
L9         1599 DUP REM L8 (3200 DUPLICATES REMOVED)

=> s l9 and (IKK or PDE)
L10           0 L9 AND (IKK OR PDE)

=> s  I-kappa kinase and GFP
L11           1 I-KAPPA KINASE AND GFP

=> d l11 ibib ab

L11  ANSWER 1 OF 1  BIOSIS  COPYRIGHT (c) 2004 The Thomson Corporation.  on STN
ACCESSION NUMBER:    2001:92225  BIOSIS
DOCUMENT NUMBER:     PREV200100092225
TITLE:               Intracellular delivery of proteins into endothelial cells
```

by membrane permeable peptide vectors.
AUTHOR(S): Baranyi, U. [Reprint author]; Schwartz, C. [Reprint author]; Kroismayr, R. [Reprint author]; Binder, B. R. [Reprint author]; Lipp, J. [Reprint author]
CORPORATE SOURCE: Vascular Biology and Thrombosis Research, University of Vienna-VIRCC, Vienna, Austria
SOURCE: Journal of Submicroscopic Cytology and Pathology, (July, 2000) Vol. 32, No. 3, pp. 473. print.
Meeting Info.: XIth International Vascular Biology Meeting. Geneva, Switzerland. September 05-09, 2000.
ISSN: 1122-9497.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 21 Feb 2001
Last Updated on STN: 12 Feb 2002

=> s phosphodiesterase? and GFP
L12 92 PHOSPHODIESTERASE? AND GFP

=> dup rem l12
PROCESSING COMPLETED FOR L12
L13 53 DUP REM L12 (39 DUPLICATES REMOVED)

=> s l13 and PDE4D
L14 0 L13 AND PDE4D

=> focus l13
PROCESSING COMPLETED FOR L13
L15 53 FOCUS L13 1-

=> s l15 and 1990-1998/py
L16 4 L15 AND 1990-1998/PY

=> d l16 1-4 ibib ab

L16 ANSWER 1 OF 4 MEDLINE on STN
ACCESSION NUMBER: 1998189355 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9512420
TITLE: Receptor-induced transient reduction in plasma membrane PtdIns(4,5)P2 concentration monitored in living cells.
AUTHOR: Stauffer T P; Ahn S; Meyer T
CORPORATE SOURCE: Department of Cell Biology, Duke University Medical Center, Durham, North Carolina 27710, USA.
CONTRACT NUMBER: GM-51457 (NIGMS)
SOURCE: Current biology : CB, **(1998 Mar 12)** 8 (6) 343-6.
Journal code: 9107782. ISSN: 0960-9822.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199805
ENTRY DATE: Entered STN: 19980609
Last Updated on STN: 19980609
Entered Medline: 19980526

AB Although phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P2) is a well-characterized precursor for the second messengers inositol 1,4,5-trisphosphate, diacylglycerol [1] and phosphatidylinositol 3,4,5-trisphosphate [2], it also interacts with the actin-binding proteins profilin and gelsolin [3], as well as with many signaling molecules that contain pleckstrin homology (PH) domains [4]. It is conceivable that stimuli received by receptors in the plasma membrane could be sufficiently strong to decrease the PtdIns(4,5)P2 concentration; this decrease could alter the structure of the cortical cytoskeleton and modulate the activity

of signaling molecules that have PH domains. Here, we tested this hypothesis by using an in vivo fluorescent indicator for PtdIns(4,5)P2, by tagging the PH domain of phospholipase C delta 1 (PLC-delta 1) with the green fluorescent protein (**GFP**-PH). When expressed in cells, **GFP**-PH was found to be enriched at the plasma membrane. Binding studies in vitro and mutant analysis suggested that **GFP**-PH bound PtdIns(4,5)P2 selectively over other phosphatidylinositol lipids. Strikingly, receptor stimulation induced a transient dissociation of **GFP**-PH from the plasma membrane, suggesting that the concentration of PtdIns(4,5)P2 in the plasma membrane was effectively lowered. This transient dissociation was blocked by the PLC inhibitor U73122 but was not affected by the phosphoinositide (PI) 3-kinase inhibitor wortmannin, suggesting that it is mostly mediated by PLC and not by PI 3-kinase activation. Overall, our studies show that PtdIns(4,5)P2 can have second messenger functions of its own, by mediating a transient dissociation of proteins anchored in the plasma membrane.

L16 ANSWER 2 OF 4 MEDLINE on STN
ACCESSION NUMBER: 93320713 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8392414
TITLE: Bubble-induced aggregation of platelets: effects of gas species, proteins, and decompression.
AUTHOR: Thorsen T; Klausen H; Lie R T; Holmsen H
CORPORATE SOURCE: Department of Anesthesiology, University of Bergen, Norway.
SOURCE: Undersea & hyperbaric medicine : journal of the Undersea and Hyperbaric Medical Society, Inc, **(1993 Jun)** 20 (2) 101-19.
Journal code: 9312954. ISSN: 1066-2936.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Space Life Sciences
ENTRY MONTH: 199308
ENTRY DATE: Entered STN: 19930826
Last Updated on STN: 19930826
Entered Medline: 19930819

AB We show that bubbles containing different gases (N2, He, Ne, Ar, or an O2-CO2-N2 mixture) are equally potent platelet agonists. The synergistic effect of different platelet antagonists does not seem to be affected by the type of gas in the bubbles. In contrast to aggregation in platelet-rich plasma (PRP), bubbles cause only a weak response in gel-filtered platelets (**GFP**), i.e., comparison of aggregation in protein-rich and protein-poor platelet suspensions may shed light on the role of different plasma proteins. Extracellular fibrinogen promotes bubble-induced platelet aggregation similar to known physiologic agonists, whereas albumin counteracts this aggregation. Bubble-induced aggregation is inhibited in **GFP**-fibrinogen by 2-deoxy-D-glucose plus antimycin A, suggesting dependency on ATP generation in the platelets and evidence for direct exposure of the "cryptic" fibrinogen receptor by bubbles. Hyperbaric compression and subsequent rapid, inadequate decompression of PRP caused little change in the aggregation response to gas bubbles and epinephrine at 1 bar, but reduced the response to ADP. Bubbles tended not to form before the surface film was broken. Pressure-induced aggregation was apparently metabolically active and not due to passive agglutination; electron microscopic studies and PRP with added glutaraldehyde did not show platelet activation, clumping, or reduced platelet count. In contrast to aggregation caused by pressure, bubble-induced aggregation in PRP at 1 bar (after treatment in the pressure chamber) was nearly completely inhibited by theophylline, a **phosphodiesterase** inhibitor that increases intracellular platelet cyclic AMP.

L16 ANSWER 3 OF 4 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1998:621301 HCAPLUS
DOCUMENT NUMBER: 129:242228

TITLE: Fluorescent protein sensors comprising fusions of green fluorescent protein and calmodulin for the detection of analytes
INVENTOR(S): Tsien, Roger Y.; Miyawaki, Atsushi
PATENT ASSIGNEE(S): The Regents of the University of California, USA
SOURCE: PCT Int. Appl., 108 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9840477	A1	19980917	WO 1998-US4978	19980313 <--
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
US 5998204	A	19991207	US 1997-818253	19970314
US 6197928	B1	20010306	US 1997-818252	19970314
AU 9867023	A1	19980929	AU 1998-67023	19980313 <--
EP 970199	A1	20000112	EP 1998-909178	19980313
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
US 2002165364	A1	20021107	US 2000-554000	20000420

PRIORITY APPLN. INFO.:

US 1997-818252	A	19970314
US 1997-818253	A	19970314
US 1997-919143	A	19970827
WO 1998-US4978	W	19980313

AB Fluorescent indicators including a binding protein moiety, a donor fluorescent protein moiety, and an acceptor fluorescent protein moiety are described. The binding protein moiety has an analyte-binding region which binds an analyte and causes the indicator to change conformation upon exposure to the analyte. The donor moiety and the acceptor moiety change position relative to each other when the analyte binds to the analyte-binding region. The donor moiety and the acceptor moiety exhibit fluorescence resonance energy transfer when the donor moiety is excited and the distance between the donor moiety and the acceptor moiety is small. The indicators can be used to measure analyte concns. is samples, such as calcium ion concns. in cells. For example, a fluorescent indicator for Ca2+ was produced by sandwiching a Xenopus calmodulin-M13 fusion between a blue (Y66H/Y145F) and a green (S65T) mutant of Aequorea green fluorescent protein (**GFP**), where M13 is the 26-residue calmodulin-binding region of myosin light-chain kinase. Chimeric protein (cameleon-1) incorporating a polyhistidine tag were expressed in Escherichia coli, purified by nickel-chelate and size-exclusion chromatog., and their fluorescence characterized. Cameleon-1 readily changes emission color by retracting and extending a long tongue (M13) into and out of the mouth of the calmodulin, the ratio of UV-excited 510 nm to 445 nm emissions increased by 70% upon binding Ca2+. Improved expression in mammalian cells is achieved by introducing mammalian codon bias into the cDNA and mutating Phe-64 to Leu, and subcellular localization signals can be added.

REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 4 OF 4 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 97:720347 SCISEARCH
THE GENUINE ARTICLE: XX399

TITLE: Stable and efficient gene transfer into the retina using an HIV-based lentiviral vector
AUTHOR: Miyoshi H; Takahashi M; Gage F H; Verma I M (Reprint)
CORPORATE SOURCE: SALK INST BIOL STUDIES, GENET LAB, POB 85800, SAN DIEGO, CA 92186 (Reprint); SALK INST BIOL STUDIES, GENET LAB, LA JOLLA, CA 92037
COUNTRY OF AUTHOR: USA
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (**16 SEP 1997**) Vol. 94, No. 19, pp. 10319-10323.
Publisher: NATL ACAD SCIENCES, 2101 CONSTITUTION AVE NW, WASHINGTON, DC 20418.
ISSN: 0027-8424.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 30

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The development of methods for efficient gene transfer to terminally differentiated retinal cells is important to study the function of the retina as well as for gene therapy of retinal diseases. We have developed a lentiviral vector system based on the HIV that can transduce terminally differentiated neurons of the brain in vivo. In this study, we have evaluated the ability of HIV vectors to transfer genes into retinal cells. An HIV vector containing a gene encoding the green fluorescent protein (**GFP**) was injected into the subretinal space of rat eyes. The **GFP** gene under the control of the cytomegalovirus promoter was efficiently expressed in both photoreceptor cells and retinal pigment epithelium. However, the use of the rhodopsin promoter resulted in expression predominantly in photoreceptor cells. Most successfully transduced eyes showed that photoreceptor cells in > 80% of the area of whole retina expressed the **GFP**. The **GFP** expression persisted for at least 12 weeks with no apparent decrease. The efficient gene transfer into photoreceptor cells by HIV vectors will be useful for gene therapy of retinal diseases such as retinitis pigmentosa.

=> s I-kappa kinase and fluorescent
L17 1 I-KAPPA KINASE AND FLUORESCENT

=> d l17 ibib ab

L17 ANSWER 1 OF 1 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 2001:92225 BIOSIS
DOCUMENT NUMBER: PREV200100092225
TITLE: Intracellular delivery of proteins into endothelial cells by membrane permeable peptide vectors.
AUTHOR(S): Baranyi, U. [Reprint author]; Schwartz, C. [Reprint author]; Kroismayr, R. [Reprint author]; Binder, B. R. [Reprint author]; Lipp, J. [Reprint author]
CORPORATE SOURCE: Vascular Biology and Thrombosis Research, University of Vienna-VIRCC, Vienna, Austria
SOURCE: Journal of Submicroscopic Cytology and Pathology, (July, 2000) Vol. 32, No. 3, pp. 473. print.
Meeting Info.: XIth International Vascular Biology Meeting. Geneva, Switzerland. September 05-09, 2000.
ISSN: 1122-9497.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 21 Feb 2001
Last Updated on STN: 12 Feb 2002

=> d his

(FILE 'HOME' ENTERED AT 11:16:21 ON 23 SEP 2004)

FILE 'MEDLINE, HCAPLUS, BIOSIS, BIOTECHDS, SCISEARCH, EMBASE' ENTERED AT 11:16:55 ON 23 SEP 2004

```
L1             0 S MODULATING I-KAPPA KINASE
L2            39 S  I-KAPPA KINASE
L3            26 DUP REM L2 (13 DUPLICATES REMOVED)
L4             0 S L3 AND CELLULAR LOCALIZATION
L5             2 S L3 AND CELLULAR
L6             7 S L3 AND INHIBITOR
L7             0 S IKK BETA AND GFP FUSION
L8          4799 S GFP FUSION
L9          1599 DUP REM L8 (3200 DUPLICATES REMOVED)
L10            0 S L9 AND (IKK OR PDE)
L11            1 S  I-KAPPA KINASE AND GFP
L12           92 S  PHOSPHODIESTERASE? AND GFP
L13           53 DUP REM L12 (39 DUPLICATES REMOVED)
L14            0 S L13 AND PDE4D
L15           53 FOCUS L13 1-
L16            4 S L15 AND 1990-1998/PY
L17            1 S  I-KAPPA KINASE AND FLUORESCENT
```

=> d l15 1-15 ibib ab

L15 ANSWER 1 OF 53 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2001:781154 HCAPLUS
DOCUMENT NUMBER: 135:327325
TITLE: Method for identifying compounds modulating intracellular distribution of **phosphodiesterase** (PDE) using live cells
INVENTOR(S): Terry, Bernard Robert; Scudder, Kurt Marshall; Bjorn, Sara Petersen; Thastrup, Ole; Almholt, Dorthe Christensen; Praestegaard, Morten
PATENT ASSIGNEE(S): Bioimage A/S, Den.
SOURCE: PCT Int. Appl., 170 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001079526	A2	20011025	WO 2001-DK264	20010411
WO 2001079526	A3	20020124		
W: AE, AG, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
EP 1276897	A2	20030122	EP 2001-923534	20010411
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
JP 2004500835	T2	20040115	JP 2001-577509	20010411
US 2003187056	A1	20031002	US 2003-257909	20030313

PRIORITY APPLN. INFO.:

DK 2000-651	A	20000417
DK 2000-849	A	20000529
WO 2000-PA651	A	20000417
WO 2000-PA849	A	20000529

AB An alternative therapeutic approach for **phosphodiesterase** 4 (PDE4) inhibition is disclosed. PDE4 dislocators, will remove the PDE4 away from the native location in the cell and thereby increase the concn. of cAMP in this location. By dislocating the PDE4, and thereby not acting directly on the catalytic, among **phosphodiesterase** inhibitors, well conserved site, the compd. will act e.g. at the binding domain of the PDE4, thereby providing isoform-specific 'inhibitors' of PDE4. The dislocation of PDE4s are visualized with fusions to **GFP**. The native location is induced by treatment with Rolipram.

L15 ANSWER 2 OF 53 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 2003:326072 BIOSIS
DOCUMENT NUMBER: PREV200300326072
TITLE: NEURAL PRECURSORS TRANSDUCED WITH FLUORESCENT POLYSIALYLTRANSFERASES ENGRAFT AND MYELINATE THE HYPOMYELINATED SHIVERER BRAIN.
AUTHOR(S): Franceschini, I. A. [Reprint Author]; Vitry, S. [Reprint Author]; Casanova, P. [Reprint Author]; Fukuda, M.; Dubois-Dalcq, M. [Reprint Author]
CORPORATE SOURCE: Neuroscience, Pasteur Institute, Paris cedex 15, France
SOURCE: Society for Neuroscience Abstract Viewer and Itinerary Planner, (2002) Vol. 2002, pp. Abstract No. 726.9. http://sfn.scholarone.com. cd-rom.
Meeting Info.: 32nd Annual Meeting of the Society for Neuroscience. Orlando, Florida, USA. November 02-07, 2002. Society for Neuroscience.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 16 Jul 2003
Last Updated on STN: 16 Jul 2003

AB To investigate whether neural cells, engineered to enhance migration, can myelinate after grafting, we retrovirally transduced neurospheres with green fluorescent protein (**GFP**) or with **GFP** fused to the glycosyltransferases PST (PST-**GFP**) and STX (STX-**GFP**).These enzymes elongate polymers of a 2->8 linked sialic acid on NCAM to synthesize PSA-NCAM to form the highly polysialylated (PSA) form of N-CAM implicated in migration. In contrast to the diffuse location of **GFP**, STX-**GFP** and PST-**GFP** chimeric proteins were located in the Golgi area of transduced neural cells which maintained PSA-NCAM expression during differentiation of 23-cyclic nucleotide 3-**phosphodiesterase** positive oligodendrocytes. The three types of genetically engineered neurosphere cells were transplanted in the lateral ventricles of newborn shiverer mice deficient for the myelin basic protein (MBP). After two months, only native **GFP** and PST-**GFP** positive cells were readily detected. However, in all grafts, well developed MBP-positive myelin patches were widely disseminated in the white matter tracts close to cerebral ventricules. Some grafted cells had even reached and myelinated the olfactory bulb. Thus engineered neural cells are able to efficiently engraft and repair white matter areas. The use of stereotaxic injections in newborn shiverer should allow to compare migration patterns/speeds between the different types of engineered cells.

L15 ANSWER 3 OF 53 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 2001:498305 BIOSIS
DOCUMENT NUMBER: PREV200100498305
TITLE: In vivo identification, characterization, and FACS purification of oligodendrocyte lineage cells using a CNP-**GFP** transgenic mouse model.
AUTHOR(S): Yuan, X. [Reprint author]; Chittajallu, R. [Reprint author]; Gallo, V. [Reprint author]
CORPORATE SOURCE: Laboratory of Cellular and Synaptic Neurophysiology,

NICHD/NIH, Bethesda, MD, USA

SOURCE: Society for Neuroscience Abstracts, (2001) Vol. 27, No. 1, pp. 938. print.
Meeting Info.: 31st Annual Meeting of the Society for Neuroscience. San Diego, California, USA. November 10-15, 2001.
ISSN: 0190-5295.

DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)

LANGUAGE: English

ENTRY DATE: Entered STN: 24 Oct 2001
Last Updated on STN: 23 Feb 2002

AB A transgenic mouse, which expresses green fluorescent protein (**GFP**) under the control of the 2'3'-cyclic nucleotide 3'-**phosphodiesterase** (CNP) promoter, was generated. Both direct fluorescence microscopy and immunohistochemistry analysis indicated that **GFP** expression in the CNS was restricted to oligodendrocyte lineage cells. At different developmental stages, **GFP**+ cells were stained with NG2, CNP, MBP and RIP antibodies, but were not stained with GFAP, TUJ1 and NeuN antibodies. The appearance of **GFP**+ oligodendrocytes could be observed as early as E10 in the developing brain and spinal cord, and in dorsal root ganglion (DRG) and neural crest cells projecting out of DRGs. During development, the morphology, migration pattern, distribution, proliferation and differentiation of **GFP**+ cells strongly resembled those of developing oligodendrocytes previously described in vivo. **GFP**+ cells were purified from transgenic brains using FACS technology. The purified cells were cultured in vitro with mitogens, and under these conditions proliferated and differentiated to oligodendrocytes. The appearance of astrocytes in cultures obtained from the FACS-sorted cells also indicated the multi-potential nature of some of the **GFP**+ cells. The direct and reliable identification of oligodendrocyte lineage cells in live and fixed tissues of the CNP-**GFP** transgenic mouse allows more detailed studies of oligodendrocyte development and other biological functions, such as cell migration and changes in physiological properties during maturation.

L15 ANSWER 4 OF 53 MEDLINE on STN

ACCESSION NUMBER: 2003341696 MEDLINE

DOCUMENT NUMBER: PubMed ID: 12873709

TITLE: Occupancy of the catalytic site of the PDE4A4 cyclic AMP **phosphodiesterase** by rolipram triggers the dynamic redistribution of this specific isoform in living cells through a cyclic AMP independent process.

AUTHOR: Terry Robert; Cheung York-Fong; Praestegaard Morten; Baillie George S; Huston Elaine; Gall Irene; Adams David R; Houslay Miles D

CORPORATE SOURCE: BioImage A/S, Moerkhoej Bygade 28, Soeborg DK-2860, Denmark.. Bob.Terry@bioimage.com

SOURCE: Cellular signalling, (2003 Oct) 15 (10) 955-71.
Journal code: 8904683. ISSN: 0898-6568.

PUB. COUNTRY: England: United Kingdom

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200404

ENTRY DATE: Entered STN: 20030723
Last Updated on STN: 20040403
Entered Medline: 20040402

AB In cells transfected to express wild-type PDE4A4 cAMP **phosphodiesterase** (PDE), the PDE4 selective inhibitor rolipram caused PDE4A4 to relocalise so as to form accretion foci. This process was followed in detail in living cells using a PDE4A4 chimera formed with Green Fluorescent Protein (**GFP**). The same pattern of behaviour was also seen in chimeras of PDE4A4 formed with various proteins and peptides, including LimK, RhoC, FRB and the V5-6His tag. Maximal PDE4A4

foci formation, occurred over a period of about 10 h, was dose-dependent on rolipram and was reversible upon washout of rolipram. Inhibition of protein synthesis, using cycloheximide, but not PKA activity with H89, inhibited foci generation. Foci formation was elicited by Ro20-1724 and RS25344 but not by either Ariflo or RP73401, showing that not all PDE4 selective inhibitors had this effect. Ariflo and RP73401 dose-dependently antagonised rolipram-induced foci formation and dispersed rolipram pre-formed foci as did the adenylyl cyclase activator, forskolin. Foci formation showed specificity for PDE4A4 and its rodent homologue, PDE4A5, as it was not triggered in living cells expressing the PDE4B2, PDE4C2, PDE4D3 and PDE4D5 isoforms as **GFP** chimeras. Altered foci formation was seen in the Deltab-LR2-PDE4A4 construct, which deleted a region within LRZ, showing that appropriate linkage between the N-terminal portion of PDE4A4 and the catalytic unit of PDE4A4 was needed for foci formation. Certain single point mutations within the PDE4A4 catalytic site (His505Asn, His506Asn and Val475Asp) were shown to ablate foci formation but still allow rolipram inhibition of PDE4A4 catalytic activity. We suggest that the binding of certain, but not all, PDE4 selective inhibitors to PDE4A4 induces a conformational change in this isoform by 'inside-out' signalling that causes it to redistribute in the cell. Displacing foci-forming inhibitors with either cAMP or inhibitors that do not form foci can antagonise this effect. Specificity of this effect for PDE4A4 and its homologue PDE4A5 suggests that interplay between the catalytic site and the unique N-terminal region of these isoforms is required. Thus, certain PDE4 selective inhibitors may exert effects on PDE4A4 that extend beyond simple catalytic inhibition. These require protein synthesis and may lead to redistribution of PDE4A4 and any associated proteins. Foci formation of PDE4A4 may be of use in probing for conformational changes in this isoform and for sub-categorising PDE4 selective inhibitors.

L15 ANSWER 5 OF 53 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2000:842156 HCAPLUS
DOCUMENT NUMBER: 134:14918
TITLE: Green fluorescent protein analogs containing ligand-binding sensor peptides for use as reporter moieties
INVENTOR(S): Tsien, Roger Y.; Baird, Geoffrey A.
PATENT ASSIGNEE(S): Regents of the University of California, USA
SOURCE: PCT Int. Appl., 94 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000071565	A2	20001130	WO 2000-US13684	20000517
WO 2000071565	C2	20020704		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 6469154	B1	20021022	US 1999-316919	19990521
US 6699687	B1	20040302	US 1999-316920	19990521
AU 2000052751	A5	20001212	AU 2000-52751	20000517
US 2002157120	A1	20021024	US 2001-999745	20011023
PRIORITY APPLN. INFO.:			US 1999-316919 A	19990521
			US 1999-316920 A	19990521

WO 2000-US13684 W 20000517

AB The present invention provides polypeptide and polynucleotides encoding fluorescent indicators having inserted within a fluorescent moiety a sensor polypeptide. The proteins are derivs. that are not normally fluorescent as a result of FRET coupling. -Binding of a ligand to the sensor results in a conformational change and an increase in fluorescence of the protein. Also provided are methods of using the fluorescent indicator. Circularly permuted fluorescent polypeptides and polynucleotides are also provided.

L15 ANSWER 6 OF 53 MEDLINE on STN
ACCESSION NUMBER: 2001204629 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11238914
TITLE: Subtype-specific translocation of the delta subtype of protein kinase C and its activation by tyrosine phosphorylation induced by ceramide in HeLa cells.
AUTHOR: Kajimoto T; Ohmori S; Shirai Y; Sakai N; Saito N
CORPORATE SOURCE: Laboratory of Molecular Pharmacology, Biosignal Research Center, Kobe University, Nada-ku, Kobe 657-8501, Japan.
SOURCE: Molecular and cellular biology, (2001 Mar) 21 (5) 1769-83. Journal code: 8109087. ISSN: 0270-7306.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200104
ENTRY DATE: Entered STN: 20010417
Last Updated on STN: 20010417
Entered Medline: 20010412

AB We investigated the functional roles of ceramide, an intracellular lipid mediator, in cell signaling pathways by monitoring the intracellular movement of protein kinase C (PKC) subtypes fused to green fluorescent protein (**GFP**) in HeLa living cells. C(2)-ceramide but not C(2)-dihydroceramide induced translocation of delta PKC-**GFP** to the Golgi complex, while alpha PKC- and zeta PKC-**GFP** did not respond to ceramide. The Golgi-associated delta PKC-**GFP** induced by ceramide was further translocated to the plasma membrane by phorbol ester treatment. Ceramide itself accumulated to the Golgi complex where delta PKC was translocated by ceramide. Gamma interferon also induced the delta PKC-specific translocation from the cytoplasm to the Golgi complex via the activation of Janus kinase and Mg(2+)-dependent neutral sphingomyelinase. Photobleaching studies showed that ceramide does not evoke tight binding of delta PKC-**GFP** to the Golgi complex but induces the continuous association and dissociation of delta PKC with the Golgi complex. Ceramide inhibited the kinase activity of delta PKC-**GFP** in the presence of phosphatidylserine and diolein in vitro, while the kinase activity of delta PKC-**GFP** immunoprecipitated from ceramide-treated cells was increased. The immunoprecipitated delta PKC-**GFP** was tyrosine phosphorylated after ceramide treatment. Tyrosine kinase inhibitor abolished the ceramide-induced activation and tyrosine phosphorylation of delta PKC-**GFP**. These results suggested that gamma interferon stimulation followed by ceramide generation through Mg(2+)-dependent sphingomyelinase induced delta PKC-specific translocation to the Golgi complex and that translocation results in delta PKC activation through tyrosine phosphorylation of the enzyme.

L15 ANSWER 7 OF 53 MEDLINE on STN
ACCESSION NUMBER: 2003169104 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12686596
TITLE: IGF-I-induced differentiation of L6 myogenic cells requires the activity of cAMP-**phosphodiesterase**.
AUTHOR: De Arcangelis Vania; Coletti Dario; Conti Marco; Lagarde Michel; Molinaro Mario; Adamo Sergio; Nemoz Georges; Naro Fabio

CORPORATE SOURCE: Dipartimento di Istologia ed Embriologia Medica, Universita di Roma La Sapienza, 00161 Roma, Italia.
SOURCE: Molecular biology of the cell, (2003 Apr) 14 (4) 1392-404. Journal code: 9201390. ISSN: 1059-1524.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200306
ENTRY DATE: Entered STN: 20030416
Last Updated on STN: 20030625
Entered Medline: 20030624

AB Inhibition of type 4 cAMP-specific **phosphodiesterase** (PDE4) activity in L6-C5 and L6-E9 abolished myogenic differentiation induced by low-serum medium and IGF-I. L6-C5 cells cultured in low-serum medium displayed a PDE4 activity higher than cells cultured in serum-free medium, a condition not sufficient to induce differentiation. In the presence of serum, PDE4D3, the major isoform natively expressed in L6-C5 cells, translocated to a Triton-insoluble fraction, which increased the PDE specific activity of the fraction, and exhibited a Mr shift typical of phosphorylation of this isoform. Furthermore, serum promoted the localization of PDE4D3 to a vesicular subcellular compartment. In L6-C5 cells, IGF-I is a stronger inducer of myogenic differentiation in the presence than in absence of serum. Its ability to trigger differentiation in the absence of serum was restored by overexpressing wild-type PDE4D3, but not a phosphorylation-insensitive mutant. This finding was confirmed in single cells overexpressing a **GFP**-PDE4D3 fusion protein by assessing nuclear accumulation of myogenin in both L6-C5 and L6-E9. Overexpression of other PDE isoforms was less efficient, confirming that PDE4D3 is the physiologically relevant **phosphodiesterase** isoform in the control of myogenesis. These results show that downregulation of cAMP signaling through cAMP-**phosphodiesterase** stimulation is a prerequisite for induction of myogenesis.

L15 ANSWER 8 OF 53 MEDLINE on STN
ACCESSION NUMBER: 2003004038 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12509985
TITLE: EGFP as a fusion partner for the expression and organic extraction of small polypeptides.
AUTHOR: Skosyrev Vitaly S; Rudenko Natalja V; Yakhnin Alexander V; Zagranichny Vasily E; Popova Lubov I; Zakharov Mikhail V; Gorokhovatsky Andrey Yu; Vinokurov Leonid M
CORPORATE SOURCE: Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Pushchino Branch, Russian Academy of Sciences, Pushchino, Moscow 142290, Russia.. vitaly@fibkh.serpukhov.su
SOURCE: Protein expression and purification, (2003 Jan) 27 (1) 55-62.
Journal code: 9101496. ISSN: 1046-5928.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200309
ENTRY DATE: Entered STN: 20030103
Last Updated on STN: 20030903
Entered Medline: 20030902

AB Green fluorescent protein (**GFP**) is widely used as an excellent reporter module of the fusion proteins. The unique structure of **GFP** allows isolation of the active fluorescent protein directly from the crude cellular sources by extraction with organic solvents. We demonstrated the stable expression of four short polypeptides fused to **GFP** in Escherichia coli cells, including antimicrobial cationic peptides, which normally kill bacteria. EGFP module protected fusion partners from the intracellular degradation and allowed the purification of the chimerical proteins by organic extraction. The nature of the

polypeptide fused to **GFP**, as opposed to the order of **GFP** and the polypeptide modules in the fusion protein, influenced the efficiency of the described purification technique.

L15 ANSWER 9 OF 53 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 2004:205530 BIOSIS
DOCUMENT NUMBER: PREV200400206046
TITLE: Isoform - dependent palmitoylation and intracellular targeting of cGMP - stimulated **phosphodiesterase** (pde2a) .
AUTHOR(S): Fretier, P. [Reprint Author]; Trinh, A.; Rujun, K.; El-Husseini, A.; Vincent, S. R.
CORPORATE SOURCE: Dept. Psychiat, Univ. British Columbia, Vancouver, BC, Canada
SOURCE: Society for Neuroscience Abstract Viewer and Itinerary Planner, (2003) Vol. 2003, pp. Abstract No. 898.2. http://sfn.scholarone.com. e-file.
Meeting Info.: 33rd Annual Meeting of the Society of Neuroscience. New Orleans, LA, USA. November 08-12, 2003. Society of Neuroscience.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 14 Apr 2004
Last Updated on STN: 14 Apr 2004

AB NO acts via cGMP to regulate ion channels, protein kinases and **phosphodiesterases**. The cGMP-stimulated **phosphodiesterase** (PDE2A) is highly expressed in brain, and provides a mechanism for NO to regulate cAMP levels in target cells. We have found using immunohistochemistry that PDE2A is widely expressed in distinct populations of central neurons. In particular it is found in some NOS neurons (mesopontine tegmentum), as well as in neurons thought to be targets of NO signaling (Purkinje cells, striatonigral and striatopallidal neurons). Our RT-PCR analysis shows that PDE2 is expressed as three distinct splice variants in brain, which differ only at the N-terminal. One isoform (PDE2A3) contains a putative myristoylation site (G2) followed by two cysteine residues at positions 5 and 11 that could be palmitoylated. The other two splice variants lack these sites for lipid modification. Using (3H)-labelled palmitate, we found that PDE2A3 is the only palmitoylated splice variant. We next examined whether protein palmitoylation occurs on both cysteines and whether it depends on protein myristoylation. We find C5S or C11S mutants are still palmitoylated, but protein palmitoylation is abolished in the C5S,C11S double mutant and in a G2A mutant. These results indicate that both cysteines are palmitoylated and this relies on prior protein myristoylation. To test whether differential palmitoylation regulates protein localization in neurons, we next examined the effect of these mutations on protein targeting in cultured neurons. We find that wild type PDE2A3-**GFP** was localized to spines, while the non-palmitoylated forms were diffusely expressed throughout the cytoplasm. Our results indicate that the different splice variants of PDE2A are targeted to distinct domains in neurons, where they can regulate local cyclic nucleotide signaling.

L15 ANSWER 10 OF 53 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 2004:196344 BIOSIS
DOCUMENT NUMBER: PREV200400196903
TITLE: Characterization of neuronal subpopulations selectively expressing green fluorescent protein in spinal cord dorsal horn and dorsal root ganglia of transgenic mice.
AUTHOR(S): Torsney, C. [Reprint Author]; Anderson, R. L. [Reprint Author]; Hatten, M. E.; Heintz, N.; MacDermott, A. B. [Reprint Author]
CORPORATE SOURCE: Dept. of Physiology and Cell. Biophysics and Ctr. for

NeuroBiol. and Behavior, Columbia Univ., New York, NY, USA
SOURCE: Society for Neuroscience Abstract Viewer and Itinerary Planner, (2003) Vol. 2003, pp. Abstract No. 260.7. http://sfn.scholarone.com. e-file.
Meeting Info.: 33rd Annual Meeting of the Society of Neuroscience. New Orleans, LA, USA. November 08-12, 2003. Society of Neuroscience.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 14 Apr 2004
Last Updated on STN: 14 Apr 2004

AB The complex neuronal circuitry of the dorsal horn of the spinal cord is as yet poorly understood. However, defining the circuits underlying the transmission of information from primary afferents to higher levels is critical to our understanding of sensory processing. In this study we have examined mice in which green fluorescent protein (**GFP**) is specifically expressed in cyclic nucleotide **phosphodiesterase** 1C (PDE1C) positive cells using bacterial artificial chromosome (BAC) technology. Using immunofluorescence we have found that **GFP** is expressed in a subpopulation of dorsal horn neurons and a subpopulation of primary sensory neurons. In the dorsal horn, the majority of **GFP** positive neurons are located in lamina I. In the dorsal root ganglia, their distribution is restricted to those subpopulations of primary sensory neurons that give rise to unmyelinated C fibres (neurofilament 200 negative). Of this population the majority bind IB4, a marker for non-peptidergic C fibres. Few **GFP** positive neurons are immunoreactive for CGRP, a marker for peptidergic C fibres. Characterization and analysis of these specific subpopulations may aid our understanding of spinal sensory processing.

L15 ANSWER 11 OF 53 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2000:810937 HCAPLUS
DOCUMENT NUMBER: 134:128044
TITLE: Fluorescent indicators for cyclic GMP based on cyclic GMP-dependent protein kinase I.alpha. and green fluorescent proteins
AUTHOR(S): Sato, Moritoshi; Hida, Naoki; Ozawa, Takeaki; Umezawa, Yoshio
CORPORATE SOURCE: Department of Chemistry School of Science, The University of Tokyo, Bunkyo-ku Tokyo, 113-0033, Japan
SOURCE: Analytical Chemistry (2000), 72(24), 5918-5924
CODEN: ANCHAM; ISSN: 0003-2700
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English

AB We describe herein fluorescent indicators for cyclic GMP (cGMP) in single living cells. cGMP-dependent protein kinase I.alpha. (PKG I.alpha.), a receptor for cGMP, was fused with blue- and red-shifted green fluorescent proteins (GFPs) to its N- and C-termini, resp. Using PKG I.alpha..DELTA.1-47, in which the dimerization domain was deleted, fluorescence resonance energy transfer between the GFPs was found to increase upon cGMP-induced conformational change in PKG I.alpha..DELTA.1-47. We demonstrated that thus-developed fluorescent indicators reversibly responded to cGMP that was produced in nitric oxide-stimulated HEK293 cells. The present genetically encoded fluorescent indicators open a way not only for understanding the dynamics of cGMP signaling in single cells and organisms but also for discovering pharmaceuticals such as isoform-specific inhibitors for **phosphodiesterases**.

REFERENCE COUNT: 34 THERE ARE 34 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 12 OF 53 MEDLINE on STN
ACCESSION NUMBER: 2003458632 MEDLINE

DOCUMENT NUMBER: PubMed ID: 12829807
TITLE: Lowering cyclic adenosine-3',5'-monophosphate (cAMP) levels by expression of a cAMP-specific **phosphodiesterase** decreases intrinsic pulsatile gonadotropin-releasing hormone secretion from GT1 cells.
AUTHOR: Yoshida Hiroshi; Beltran-Parrazal Luis; Butler Paul; Conti Marco; Charles Andrew C; Weiner Richard I
CORPORATE SOURCE: Department of Obstetrics, Gynecology and Reproductive Sciences, University of California San Francisco, San Francisco, California 94143, USA.
CONTRACT NUMBER: HD08924 (NICHD)
HD41996 (NICHD)
SOURCE: Molecular endocrinology (Baltimore, Md.), (2003 Oct) 17 (10) 1982-90.
Journal code: 8801431. ISSN: 0888-8809.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200406
ENTRY DATE: Entered STN: 20031002
Last Updated on STN: 20040624
Entered Medline: 20040622

AB Pulsatile GnRH secretion is an intrinsic property of GnRH neurons. Since increases in cAMP levels increase excitability and GnRH secretion in the GT1-1 GnRH cell line, we asked whether cAMP levels play a role in timing excitability and intrinsic pulsatile GnRH secretion. The expression of the cAMP-specific **phosphodiesterase** (PDE4D1) was used in a genetic approach to lower cAMP levels. Cells were infected with an adenovirus vector (Ad) expressing PDE4D1 (PDE-Ad), or for controls with an empty Ad (Null-Ad) or an Ad expressing green fluorescent protein (**GFP**-Ad). Infection with the PDE-Ad significantly inhibited forskolin-induced increases in cAMP production, GnRH secretion, and Ca2+ oscillations. Infection of GT1-1 cells with the PDE-Ad vs. **GFP** -Ad or Null-Ad controls significantly decreased spontaneous Ca2+ oscillations and inhibited the frequency of GnRH pulses. These data support the hypothesis that the level of cAMP in GT1 neurons is a component of the biological clock timing neuron excitability and pulsatile GnRH secretion. Genetically targeted expression of PDE4D1 represents a powerful approach to study the role of cAMP levels in specific populations of neurons in transgenic animals.

L15 ANSWER 13 OF 53 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 2002:3981 BIOSIS
DOCUMENT NUMBER: PREV200200003981
TITLE: Oligodendrocyte progenitors express purinergic receptors.
AUTHOR(S): Haak, L. L. [Reprint author]; Stevens, B. [Reprint author]; Porta, S. [Reprint author]; Yuan, X. [Reprint author]; Gallo, V. [Reprint author]; Fields, R. D. [Reprint author]
CORPORATE SOURCE: Lab for Cellular and Synaptic Neurophysiology, NIH, Bethesda, MD, USA
SOURCE: Society for Neuroscience Abstracts, (2001) Vol. 27, No. 2, pp. 2388. print.
Meeting Info.: 31st Annual Meeting of the Society for Neuroscience. San Diego, California, USA. November 10-15, 2001.
ISSN: 0190-5295.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 28 Dec 2001
Last Updated on STN: 25 Feb 2002

AB Previous work in our lab has shown that ATP release from neurons can regulate cell proliferation and differentiation in Schwann cells (Stevens

and Fields, 2000). Little is known about purinergic receptors in oligodendrocytes. We used live-cell Ca2+ imaging in fluo-3 or indo-1 loaded oligodendrocyte progenitor cells (OPCs) to determine whether purinergic receptors may be involved in oligodendrocyte development. We found evidence for both P1 (adenosine) and P2 (ATP) receptors in OPCs. In purified cultures of OPCs (n=127), almost half showed a robust Ca2+ increase to the P2 agonists 2-MeS-ATP (100muM), and 33% to gammaS-ATP (100muM). 22% of OPCs have robust intracellular Ca2+ responses to the general P1 receptor agonist NECA (100muM), and less than 3% of OPs showed a Ca2+ response to adenosine (100muM). We prepared acutely isolated OPCs from neonatal cyclic nucleotide **phosphodiesterase** (CNP)-**GFP** transgenic mice, in which only cells of the oligodendrocyte lineage express **GFP**. We found that **GFP**-positive cells showed Ca2+ responses to NECA and 2-MeS-ATP, strongly suggesting that OPCs express purinergic receptors in vivo. In co-cultures of dorsal root ganglion neurons and OPCs, electrical stimulation rapidly evoked transient intracellular Ca2+ rises in OPCs. We are using antagonists for P1/P2 and glutamate receptors to determine which receptor systems mediate this response.

L15 ANSWER 14 OF 53 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 2001:257978 BIOSIS
DOCUMENT NUMBER: PREV200100257978
TITLE: Regulation of expression and subcellular localization of **phosphodiesterase** 4B2.
AUTHOR(S): Nazarian, Steven H. [Reprint author]; Strathdee, Craig A. [Reprint author]; Madrenas, Joaquin [Reprint author]
CORPORATE SOURCE: John P. Robarts Research Institute, 100 Perth Drive, London, Ontario, N6A 5K8, Canada
SOURCE: FASEB Journal, (March 8, 2001) Vol. 15, No. 5, pp. A1040. print.
Meeting Info.: Annual Meeting of the Federation of American Societies for Experimental Biology on Experimental Biology 2001. Orlando, Florida, USA. March 31-April 04, 2001.
CODEN: FAJOEC. ISSN: 0892-6638.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 30 May 2001
Last Updated on STN: 19 Feb 2002

AB **Phosphodiesterases** (PDEs) comprise a large family of enzymes that regulate cell function by hydrolyzing cGMP and/or cAMP into 5'GMP and/or 5'AMP. Of the seven PDE subfamilies, three (PDE3, 4 and 7) have been described in T-cells, where they are required for optimal activation. We have recently reported that the PDE4B2 isoform has four potential myristylation sites and this correlates with association with the T cell receptor complex and its selective tyrosine phosphorylation following ligation of this receptor. To study the basis of subcellular localization of this enzyme isoform, we have developed a retroviral-based, gene transfer system involving expression of green fluorescence protein - PDE4B2 cDNA fusion (gfpPDE4B2). We found that the 5' untranslated region (UTR) of the full length PDE4B2 cDNA contains three translation initiation sites which might, if utilized, result in premature translation termination. When expressed in HEK293 cells, the gfpPDE4B2 cDNA with intact 5' UTR leads to very low levels of expression of PDE4B2-**GFP** . In contrast, expression of the gfpPDE4B2 cDNA without the three upstream translation starting sites leads to a significant increase of expression of gfpPDE4B2. In addition, expression of the gfpPDE4B2 cDNA leads to cell death that is prevented by a PDE4 inhibitor (rolipram - 10muM). When the subcellular localization of the gfpPDE4B2 isoform was examined with confocal microscopy, we observed that it localizes to a vacuolar compartment and in the cell membrane. These results are currently being reproduced using a human T cell line that does not express PDE4B. In summary, we have developed an expression system to study PDE4B

expression and function. Preliminary results indicate that expression of high levels of PDE4B2 may lead to cell death, supporting a role of this enzyme in the regulation of cell survival.

L15 ANSWER 15 OF 53 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 97:720347 SCISEARCH
THE GENUINE ARTICLE: XX399
TITLE: Stable and efficient gene transfer into the retina using an HIV-based lentiviral vector
AUTHOR: Miyoshi H; Takahashi M; Gage F H; Verma I M (Reprint)
CORPORATE SOURCE: SALK INST BIOL STUDIES, GENET LAB, POB 85800, SAN DIEGO, CA 92186 (Reprint); SALK INST BIOL STUDIES, GENET LAB, LA JOLLA, CA 92037
COUNTRY OF AUTHOR: USA
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (16 SEP 1997) Vol. 94, No. 19, pp. 10319-10323.
Publisher: NATL ACAD SCIENCES, 2101 CONSTITUTION AVE NW, WASHINGTON, DC 20418.
ISSN: 0027-8424.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 30

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The development of methods for efficient gene transfer to terminally differentiated retinal cells is important to study the function of the retina as well as for gene therapy of retinal diseases. We have developed a lentiviral vector system based on the HIV that can transduce terminally differentiated neurons of the brain in vivo. In this study, we have evaluated the ability of HIV vectors to transfer genes into retinal cells. An HIV vector containing a gene encoding the green fluorescent protein (**GFP**) was injected into the subretinal space of rat eyes. The **GFP** gene under the control of the cytomegalovirus promoter was efficiently expressed in both photoreceptor cells and retinal pigment epithelium. However, the use of the rhodopsin promoter resulted in expression predominantly in photoreceptor cells. Most successfully transduced eyes showed that photoreceptor cells in > 80% of the area of whole retina expressed the **GFP**. The **GFP** expression persisted for at least 12 weeks with no apparent decrease. The efficient gene transfer into photoreceptor cells by HIV vectors will be useful for gene therapy of retinal diseases such as retinitis pigmentosa.

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(FILE 'HOME' ENTERED AT 11:16:21 ON 23 SEP 2004)

FILE 'MEDLINE, HCAPLUS, BIOSIS, BIOTECHDS, SCISEARCH, EMBASE' ENTERED AT 11:16:55 ON 23 SEP 2004

L1 0 S MODULATING I-KAPPA KINASE
L2 39 S I-KAPPA KINASE
L3 26 DUP REM L2 (13 DUPLICATES REMOVED)
L4 0 S L3 AND CELLULAR LOCALIZATION
L5 2 S L3 AND CELLULAR
L6 7 S L3 AND INHIBITOR
L7 0 S IKK BETA AND GFP FUSION
L8 4799 S GFP FUSION
L9 1599 DUP REM L8 (3200 DUPLICATES REMOVED)
L10 0 S L9 AND (IKK OR PDE)
L11 1 S I-KAPPA KINASE AND GFP
L12 92 S PHOSPHODIESTERASE? AND GFP
L13 53 DUP REM L12 (39 DUPLICATES REMOVED)
L14 0 S L13 AND PDE4D

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L15           53 FOCUS L13 1-
L16            4 S L15 AND 1990-1998/PY
L17            1 S  I-KAPPA KINASE AND FLUORESCENT

=> log y
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                    95.62      95.83

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)          SINCE FILE      TOTAL
                                                       ENTRY    SESSION
CA SUBSCRIBER PRICE                                    -4.90      -4.90

STN INTERNATIONAL LOGOFF AT 11:32:56 ON 23 SEP 2004
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L8: Entry 7 of 7 File: USPT Feb 11, 2003

US-PAT-NO: 6518021
DOCUMENT-IDENTIFIER: US 6518021 B1

TITLE: Method for extracting quantitative information relating to an influence on a cellular response

DATE-ISSUED: February 11, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Thastrup; Ole	Birkerod			DK
Bj.o slashed.rn; Sara Petersen	Lyngby			DK
Tullin; Soren	Soborg			DK
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Scudder; Kurt	Virum			DK

US-CL-CURRENT: 435/6; 435/320.1, 435/325, 435/354, 435/357, 435/358, 435/365, 435/366, 435/367, 536/23.1, 536/23.5, 800/13

CLAIMS:

What is claimed is:

1. A method for detecting intracellular translocation of a component of an intracellular pathway affecting intracellular processes comprising: (a) culturing one or more cells containing a nucleotide sequence coding for a hybrid polypeptide comprising a luminophore linked to the component under conditions permitting expression of the nucleotide sequence, (b) incubating the cell or cells with a substance to be screened for biological function or biological effect, and (c) measuring the light emitted from the luminophore in the incubated cell or cells and determining the result or variation with respect to the emitted light from said luminophore, such variation being indicative of the translocation of the component in said cell or cells.

2. A method for detecting intracellular translocation of a component of an intracellular pathway affecting intracellular processes comprising: (a) culturing one or more cells containing a nucleotide sequence coding for a hybrid polypeptide comprising a luminophore linked to the component under conditions permitting expression of the nucleotide sequence, (b) incubating the cell or cells with a substance to be screened for biological function or biological effect, and (c) extracting quantitative information relating to the translocation of said component by recording variation in spatially distributed light emitted from said luminophore, such variation being indicative of the translocation of the component in said cell or cells.

3. A method according to claim 2, wherein the quantitative information

relating to the translocation of the component is extracted from the recording or recordings according to a predetermined calibration.

4. A method according to claim 1 or 2, wherein the substance to be screened for biological function or biological effect is a chemical substance.

5. A method according to claim 1 or 2, wherein the substance is a substance whose affect on an intracellular pathway is to be determined.

6. A method according to claim 1 or 2, wherein the step (c) is made at a single point in time after the application of the substance.

7. A method according to claim 1 or 2, wherein the step (c) is made at two points in time, one point being before, and the other point being after the application of the substance.

8. A method according to claim 1 or 2, wherein the step (c) is performed at a series of points in time, in which the application of the substance occurs at some time after the first time point in the series of recordings, the recording being performed within a predetermined time spacing from about 0.1 seconds to 1 hour over a time span of from 1 second to 12 hours.

9. A method according to claim 8, wherein the predetermined time spacing from 1 to 60 seconds.

10. A method according to claim 8, wherein the predetermined time spacing from 1 to 30 seconds.

11. A method according to claim 8, wherein the predetermined time spacing from 1 to 10 seconds.

12. A method according to claim 8, wherein the time span is from 10 seconds to 1 hour.

13. A method according to claim 8, wherein the time span is from 60 seconds to 30 minutes.

14. A method according to claim 8, wherein the time span is from 60 seconds to 20 minutes.

15. A method according to claim 2, wherein the cell or cells is/are fixed at a point in time after the application of the substance at which the response has been predetermined to be significant, and the recording is made at an arbitrary later time.

16. A method according to claim 1 or 2, wherein the luminophore is a luminophore which is capable of being translocated in a manner which is physiologically relevant to the degree of the substance.

17. A method according to claim 1 or 2, wherein the luminophore is a luminophore which is capable of associating with a component which is capable of being translocated in manner which is physiologically relevant to the degree of the substance.

18. A method according to claim 1 or 2, wherein the luminophore is a luminophore which is capable of being translocated in a manner which is

experimentally determined to be correlated to the degree of the substance.

19. A method according to claim 1 or 2, wherein the luminophore is a luminophore which is capable of being translocated, by modulation of the intracellular pathway, in substantially the same manner as the at least one component of the intracellular pathway.

20. A method according to claim 1 or 2, wherein the luminophore is a luminophore which is capable of being quenched upon spatial association with a component which is translocated by modulation of the pathway, the quenching being measured as a decrease in the intensity of the luminescence.

21. A method according to claim 1 or 2, wherein the variation or result with respect to the spatially distributed light emitted by the luminophore is detected by a change in the resonance energy transfer between the luminophore and another luminescent entity capable of delivering energy to the luminophore, each of which has been selected or engineered to become part of, bound to or associated with particular components of the intracellular pathway, and one of which undergoes translocation in response to the influence, thereby changing the amount of resonance energy transfer, the change in the resonance energy transfer being measured as a change in the intensity of emission from the luminophore.

22. A method according to claim 21, wherein the change in the intensity of the emission from the luminophore is sensed by a single channel photodetector which responds only to the average intensity of the luminophore in a non-spatially resolved fashion.

23. A method according to claim 1, wherein the property of the light being recorded is intensity, fluorescence lifetime, polarization, wavelength shift, or other property which is modulated as a result of the underlying cellular response.

24. A method according to claim 1 or 2, wherein the recording of the spatially distributed light is performed using a recording system which records the spatial distribution of a recordable property of the light in the form of an ordered array of values.

25. A method according to claim 24, wherein the recording of the spatial distribution of the recordable property of the light is performed using a charge transfer device or a vacuum tube device.

26. A method according to claim 25, wherein the charge transfer device is a CCD array.

27. A method according to claim 25, wherein the vacuum tube device is a vidicon tube.

28. A method according to claim 1 or 2, wherein the light to be measured passes through a filter which selects the desired component of the light to be measured and rejects other components.

29. A method according to claim 1 or 2, wherein the step (c) is performed by fluorescence microscopy.

30. A method according to claim 2, wherein the recording of the variation or result with respect to light emitted from the luminophore is performed by

recording the spatially distributed light as one or more digital images, and processing of the recorded variation to reduce it to one or more numbers representative of the degree of redistribution comprises a digital image processing procedure or combination of digital image processing procedures.

31. A method according to claim 1 or 2, wherein the intracellular pathway is an intracellular signaling pathway.

32. A method according to claim 1 or 2, wherein the luminophore is a fluorophore.

33. A method according to claim 1 or 2, wherein the luminophore is a Green Fluorescent Protein (GFP).

34. A method according to claim 33, wherein the GFP is selected from the group of GFPs having the F64L mutation.

35. A method according to claim 34, wherein the GFP is a GFP variant selected from the group consisting of F64L-GFP, F64L-Y66H-GFP, F64L-S65T-GFP, and EGFP.

36. A method for detecting intracellular translocation of a biologically active polypeptide affecting intracellular processes comprising: a) culturing one or more cells containing a nucleotide sequence coding for a hybrid polypeptide comprising a luminophore linked to a biologically active polypeptide under conditions permitting expression of the nucleotide sequence, b) incubating the cell or cells with a substance to be screened for biological function or biological effect, c) measuring the light emitted by the luminophore in the incubated cell or cells and determining the result or variation with respect to the emitted light, such result or variation being indicative of the translocation of a biologically active polypeptide in said cell or cells, and d) measuring the effect of said substance on the inhibition/activation of enzymatic activity of said biologically active polypeptide.

37. A method according to claim 1, 2 or 36, wherein the nucleotide sequence is a DNA sequence.

38. A method according to claim 1, 2 or 36 wherein the biological function or biological effect is an activation.

39. A method according to claim 1, 2 or 36, wherein the biological function or biological effect is a deactivation.

40. A method according to claim 36, wherein the emitted light of the cell or cells is measured prior to the incubation of the cells or cells with said substance, and the result or variation determined in step (c) is a change in the emitted light compared to the emitted light measured prior to the incubation of the cell or cells with said substance.

41. A method according to claim 36, wherein the intracellular processes are intracellular signalling pathways.

42. A method according to claim 36, wherein the change in the emitted light measured in step (c) comprises determining a change in the spatial distribution of the emitted light.

43. A method according to claim 1, 2 or 36, wherein the cell or cells is/are a

mammalian cell/mammalian cells which, during the time peroid over which the influence is observed, is/are incubated at a temperature of 30.degree. C. or above.

44. A method according to claim 43, wherein the cells or cells is/are incubated at a temperature of from 32.degree. C. to 39.degree. C.

45. A method according to claim 43, wherein the cells or cells is/are incubated at a temperature of from 35.degree. C. to 38.degree. C.

46. A method according to claim 43, wherein the cell or cells is/are incubated at a temperature of about 37.degree. C.

47. A method according to claim 1, 2 or 36, wherein at least one cell is part of a matrix of identical or non-identical cells.

48. A method according to claim 1, 2 or 36, wherein the cell or cells is/are selected from the group consisting of fungal cells, invertebrate cells and vertebrate cells.

49. A method according to claim 48, wherein the fungal cell or cells is/are a yeast cell.

50. A method according to claim 48, wherein the invertebrate cell or cells is/are an insect cell.

51. A method according to claim 48, wherein the vertebrate cell or cells is/are a mammalian cell.

52. A method according to claim 36, wherein the fusion polypeptide comprising a biologically active polypeptide affecting intracellular processes and a luminophore is encoded for by a nucleic acid construct wherein the construct is not a construct coding for a fusion polypeptide in which the biologically active polypeptide is not selected from the group consisting of PKC-alpha, PKC-gamma and PKC-epsilon.

53. A method according to claim 1, 2 or 36, in which the method of the invention is used as a screening program.

54. A method according to claim 1, 2 or 36, wherein the method is a screening program for the identification of a biologically active substance that directly or indirectly affects an intracellular signaling pathway and is potentially useful as a medicament, wherein the result of the individual measurement of each substance being screened which indicates its potential biological activity is based on measurement of the redistribution of spatially resolved luminescence in living cells and which undergoes a change in distribution upon activation of an intracellular signaling pathway.

55. A method according to claim 1, 2 or 36, wherein the method is a screening program for the identification of a biologically toxic substance as defined herein that exerts its toxic effect by interfering with an intracellular signaling pathway, wherein the result of the individual measurement of each substance being screened which indicates its potential biologically toxic activity is based on measurement of the redistribution of said luminophore in living cells and which undergoes a change in distribution upon activation of an intracellular signaling pathway.

56. A method according to claim 1, 2 or 36, wherein the luminophore is used in backtracking of signal transduction pathways.

57. A method according to claim 1, 2 or 36 of identifying a drug target among the group of biologically active polypeptides which are components of intracellular signalling pathways.

58. A method according to claim 36, wherein the luminophore is a fluorophore.

59. A method according to claim 36, wherein the luminophore is a Green Fluorescent Protein (GFP).

60. A method according to claim 59, wherein the GFP is selected from the group of GFPs having the F64L mutation.

61. A method according to claim 60, wherein the GFP is a GFP variant selected from the group consisting of F64L-GFP, F64L-Y66H-GFP, F64L-S65T-GFP, and EGFP.

62. A method according to claim 36, wherein the step (c) is made at a single point in time after the application of the substance.

63. A method according to claim 36, wherein the step (c) is made at two points in time, one point being before, and the other point being after the application of the substance.

64. A method according to claim 36, wherein the step (c) is performed at a series of points in time, in which the application of the substance occurs at some time after the first time point in the series of recordings, the recording being performed within a predetermined time spacing from about 0.1 seconds to 1 hour over a time span of from 1 second to 12 hours.

65. A method according to claim 64, wherein the predetermined time spacing from 1 to 60 seconds.

66. A method according to claim 64, wherein the predetermined time spacing from 1 to 30 seconds.

67. A method according to claim 64, wherein the predetermined time spacing from 1 to 10 seconds.

68. A method according to claim 64, wherein the time span is from 10 seconds to 1 hour.

69. A method according to claim 64, wherein the time span is from 60 seconds to 30 minutes.

70. A method according to claim 64, wherein the time span is from 60 seconds to 20 minutes.

71. A method according to claim 36, wherein the cell or cells is/are fixed at a point in time after the application of the substance at which the response has been predetermined to be significant, and the recording is made at an arbitrary later time.

72. A method according to claim 36, wherein the luminophore is a luminophore which is capable of being translocated in a manner which is physiologically relevant to the degree of the substance.

73. A method according to claim 36, wherein the luminophore is a luminophore which is capable of associating with a biologically active polypeptide which is capable of being translocated in manner which is physiologically relevant to the degree of the substance.

74. A method according to claim 36, wherein the luminophore is a luminophore which is capable of being translocated in a manner which is experimentally determined to be correlated to the degree of the substance.

75. A method according to claim 36, wherein the luminophore is a luminophore which is capable of being translocated, by modulation of the intracellular pathway, in substantially the same manner as the at least one component of the intracellular pathway.

76. A method according to claim 36, wherein the luminophore is a luminophore which is capable of being quenched upon spatial association with a component which is translocated by modulation of the pathway, the quenching being measured as a decrease in the intensity of the luminescence.

77. A method according to claim 36, wherein the variation or result with respect to the spatially distributed light emitted by the luminophore is detected by a change in the resonance energy transfer between the luminophore and another luminescent entity capable of delivering energy to the luminophore, each of which has been selected or engineered to become part of, bound to or associated with particular components of the intracellular pathway, and one of which undergoes translocation in response to the influence, thereby changing the amount of resonance energy transfer, the change in the resonance energy transfer being measured as a change in the intensity of emission from the luminophore.

78. A method according to claim 77, wherein the change in the intensity of the emission from the luminophore is sensed by a single channel photodetector which responds only to the average intensity of the luminophore in a non-spatially resolved fashion.

79. A method according to claim 36, wherein the property of the light being recorded is intensity, fluorescence lifetime, polarization, wavelength shift, or other property which is modulated as a result of the underlying cellular response.

80. A method according to claim 36, wherein the recording of the spatially distributed light is performed using a recording system which records the spatial distribution of a recordable property of the light in the form of an ordered array of values.

81. A method according to claim 36, wherein the recording of the spatial distribution of the recordable property of the light is performed using a charge transfer device or a vacuum tube device.

82. A method according to claim 81, wherein the charge transfer device is a CCD array.

83. A method according to claim 81, wherein the vacuum tube device is a vidicon tube.

84. A method according to claim 36, wherein the light to be measured passes through a filter which selects the desired component of the light to be measured and rejects other components.

85. A method according to claim 36, wherein the step (c) is performed by fluorescence microscopy.

86. A method according to claim 52, wherein the intracellular process is an intracellular signalling pathway.

87. A method according to claim 52 or 86, wherein the biologically active polypeptide is selected from the group consisting of a protein kinase, a phosphatase, a transcription factor and a protein associated with the cytoskeletal network which change cellular localization upon activation.

88. A method according to claim 1 or 2, wherein the fusion polypeptide comprising a luminophore linked to a component of an intracellular pathway affecting intracellular processes is encoded for by a nucleic acid construct.

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☐	L7	IKK with GFP	0
☐	L6	IKK and GFP	91
☐	L5	IKK	761
☐	L4	I-kappa kinase	4
☐	L3	kappa kinase with green fluoroscent protein	0
☐	L2	kappa kinase with green fluoroscent protein.clm.	0
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